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EFFECT OF A CONTRACEPTIVE STEROID COMBINATION ON THE "SERUM ACTIVATION" OF LIPOPROTEIN LIPASE IN RATS

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Abstract—Our investigation on serum obtained from control rats and from rats treated with a steroid contraceptive drug (SCD) showed that serum from SCD treated rats had a lower activating capacity for lipoprotein lipase from rat epididymal adipose tissue. A similar result was observed when HDL apolipoproteins obtained from serum of these two groups of rats were tested against a lipoprotein lipase preparation. The data suggest a deficiency or an alteration of some serum cofactors (probably apolipoproteins of the C group). The observation that chylomicrons from SCD treated rats were slightly poorer substrate for lipoprotein lipase than those from control rats supports the hypothesis of a cofactor deficiency rather than a transfer of apolipoproteins from HDL to chylomicrons.

The mechanism by which the use of steroid contraceptive drugs (SCD) results in a rise of plasma triglycerides in some experimental animals and often in women is not yet fully elucidated [1, 2].

Hepatic triglyceride synthesis as well as plasma removal seem to be involved. Results from kinetic studies suggest that plasma removal is increased by SCD treatment, but findings on lipoprotein lipase in rats and post heparin lipoprotein lipase activity (in women) do not give a conclusive appraisal of the effect of SCD on these enzymes which are generally regarded as the key factors in the clearance of triglycerides from plasma [3–12]. Studies on the influence of SCD on triglyceride hydrolysis have mainly centered on the lipoprotein lipase itself while no attention has been paid to the serum factors responsible for its activation.

The data reported in this study provide evidence that the administration of SCD to female rats reduces the serum's capacity to activate lipoproteinlipase and that this defect probably lies in the apolipoprotein moiety.

MATERIALS AND METHODS

Animals. Adult female rats (CD, Charles River, Italy), of body wt 200 ± 10 g, received daily an oral dose of lynestranol plus mestranol for 30 days (5 + 0.3 mg/kg body wt). Controls received the same amount of vehicle (corn oil 0.1 ml/rat).

The animals, which had food available during the experiment, were killed (always at 11 a.m.) 18 hr after the last administration. Their sera were collected for lipoprotein lipase assay or for preparation of apolipoproteins (apo-HDL). In some instances female rats, treated as above, received olive oil (20 ml/kg), 2 hr before killing so that their serum could be used for the preparation of chylomicrons.

Lipoprotein lipase assay. Lipoprotein lipase was obtained from epididymal adipose tissue of 100–125 g male rats as described by Cherkes and Gordon [13].

Two substrates were assayed: (a) coconut oil emulsion (Ediol 4%) and (b) chylomicrons obtained from female rats treated or not with the contraceptive combination.

The assay tubes containing 50 μl of substrate (final triglyceride concentration from 0.25 to 2 mg/ml), 50 μl of fresh serum from SCD or control animals and 400 μl bovine serum albumin 10% in buffer NH_4OH–NH_4Cl (0.025 M, pH 8.6) were preincubated at 37° for 30 min. After addition of 500 μl of medium containing the lipoprotein lipase the mixture was incubated for 60 min at 37°. The lipase activity was determined titrimetrically as the rate of release of free fatty acid (FFA) from triglycerides after subtraction of basal values. FFA were measured according to Trout [14].

In one experiment different amounts of serum ranging from 25 to 150 μl were added; molarity of buffer and albumin concentrations were adjusted in order to have similar final concentrations. In another ex-

Table 1. Effect of serum from control and SCD treated rats on adipose tissue lipoprotein lipase in presence of different amounts of substrate

Substrate triglyceride mg/ml	FFA μEq/g/hr ± S.E. Control serum	SCD serum
1	5.97 ± 0.08	6.91 ± 0.10
2	8.56 ± 0.09	7.83 ± 0.68
3	11.29 ± 0.21	8.57 ± 0.32*
4	13.53 ± 0.62	7.96 ± 0.01*
6	12.75 ± 0.24	8.80 ± 0.08*
8	15.83 ± 0.08	9.17 ± 0.29*

The triglyceride substrate was a synthetic coconut oil emulsion (EDIOL). Serum was added at the concentration of 50 μl/ml. SCD serum was obtained from female rats treated with lynestranol plus mestranol combination 5 + 0.3 mg/kg daily for 30 days.

Lipoprotein lipase activity was equal in each tube assay. Each figure is the mean of four determinations.

* $P < 0.01$ against respective control group according to Student's 't' test.

Table 2. Effect of different amounts of serum obtained from control or SCD treated rats on adipose tissue lipoprotein lipase activity

Triglyceride mg/ml	Serum μl	FFA μEq/l/hr ± S.E. Control serum	SCD serum
4	25	4.86 ± 0.19	3.41 ± 0.23
4	50	11.45 ± 0.86	6.09 ± 0.57*
4	100	15.76 ± 0.31	11.86 ± 0.53*
4	150	17.39 ± 0.76	13.30 ± 0.44*

Legend as in Table 1.
* $P < 0.01$ against respective control group according to Student's 't' test.

Table 3. Activation of lipoprotein lipase with apo-HDL obtained from serum of control of SCD treated rats

apo-HDL mg protein/ml	Triglyceride	FFA μEq/g/hr ± S.E. Control apo-HDL	SCD apo-HDL
0.125	2	12.00 ± 0.73	3.36 ± 0.4*
0.250	2	17.91 ± 0.60	2.85 ± 0.3*
0.500	2	20.77 ± 0.02	7.85 ± 0.6*

Legend as in Table 1.
* $P < 0.01$ against respective control group according to Student's 't' test.

Table 4. Hydrolysis of triglyceride-chylomicrons obtained from control and SCD treated rats

Chylomicrons triglyceride mg/ml	Control serum μl	FFA μEq/g/hr ± S.E. Control chylomicrons	SCD chylomicrons
0.25	—	2.50 ± 0.12	1.85 ± 0.16*
0.5	—	5.36 ± 0.18	3.75 ± 0.13*
1.0	—	10.34 ± 0.14	8.12 ± 0.22*
2.0	—	17.24 ± 0.09	15.88 ± 0.13*
3.0	—	23.29 ± 0.35	20.67 ± 0.16*
3.0	50	22.02 ± 0.30	22.76 ± 0.70

Lipoprotein lipase activity was similar in each tube assay.
* $P < 0.01$ against respective control group according to Student's 't' test.

periment serum was substituted with different amounts of apo-HDL.

Chylomicron preparation. Serum of rats pretreated as described above was centrifuged at 8700 *g* for 15 min. Chylomicrons were separated, resuspended in NaCl 0.15 M plus EDTA 0.005%, and centrifuged twice. Finally they were suspended in albumin, $NH_4OH \cdot NH_4Cl$ buffer, to obtain suitable concentrations of triglycerides. Triglycerides were measured according to Van Handel and Zilversmit [15].

Preparation of apolipoproteins. Serum VLDL, LDL and HDL were separated according to Havel's method [16]. Lipoprotein fractions were lyophilized and subsequently delipidized as described by Shore and Shore [17]. The protein content of apolipoproteins was determined according to Lowry [18].

RESULTS

Results in Table 1 show that for a fixed amount of lipoprotein lipase the rate of hydrolysis of triglycerides depended both on the concentration of triglycerides and on the source of serum added to the assay medium.

The rate of lipolysis was lower when serum, obtained from rats treated with the estrogen–progestinic combination, was used instead of control serum. The difference in the rate of hydrolysis was related to the substrate concentration, the maximum being reached at 4 mg/ml. When a fixed amount of substrate was utilized, as reported in Table 2, the rate of lipolysis increased in relation to the amount of serum added, but this increase was less marked when the serum was obtained from SCD treated rats.

Since the activation of lipoprotein lipase by serum is related to the presence in the serum of apolipoproteins of the C group (which are present in HDL as well as in VLDL and chylomicrons), a lipoprotein lipase preparation was activated by apo-HDL from serum either of normal or of SCD treated female rats (Table 3). Again, the lipoprotein lipase activation was markedly lower when the apo-HDL were obtained from serum of treated rather than from control rats.

To check whether this finding might be of some relevance in the mechanism of hypertriglyceridemia induced by the administration of SCD, chylomicrons, which are physiological substrates *in vivo* for lipoprotein lipase, were used. Results in Table 4 show that the rate of hydrolysis of triglycerides was slightly higher when chylomicrons from normal rats were used, chylomicrons from rats treated with SCD being a relatively poorer substrate. However, when serum

of control rats was added to the assay medium, the activity of lipoprotein lipase was similar in the two experimental conditions.

DISCUSSION

The present work shows that in lipoprotein lipase activity the rate of triglyceride hydrolysis depends, among other factors on the source of serum used for substrate (Ediol) activation. It was lower when the serum was drawn from female rats chronicallv treated with a progestin–estrogen combination, than when drawn from control female rats. This effect might be due to a deficiency of some lipoprotein lipase cofactors in the serum, or to the presence of an inhibitor. The latter hypothesis seems unlikely since the rate of lipolysis increased with the amount of serum added to the assay medium.

The observation that apo-HDL from sera of rats treated with the lynestranol-mestranol combination, activated lipoproteinlipase of adipose tissue to a lesser extent than apo-HDL from control serum. seems to support the hypothesis of a deficiency or alteration of the cofactors, probably apolipoproteins belonging to the C group.

Recent studies have shown that formation of the enzyme-substrate combination required for hydrolysis of triglyceride, depends on the presence of C group apolipoproteins (apo CII for adipose tissue lipoprotein lipase) which are components of apo-HDL [19–21]. Studies by Havel *et al.* [22] have shown that in man after the ingestion of a fat rich meal the "activator protein" was transferred from HDL to chylomicrons. For this reason chylomicrons produced by a triglyceride load given by tube feeding were utilized in additional experiments. It was found that chylomicrons obtained from SCD treated rats, were slightly poorer substrates for lipoprotein-lipase activity than those obtained from normal rats.

Whether this effect also depends on a deficiency of apolipoproteins has still to be proved, but it is significant that the addition of control serum abolished the difference between the two sources of chylomicrons. Whether a defiency of lipoprotein lipase activation also occurs in women taking steroid contraceptives and whether this is relevant to the onset of hypertriglyceridemia might be worth investigating in view of a possible relation between endothelial lipoprotein lipase activity and atherosclerosis [23].

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